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SUMMARY STATEMENT
(Privileged Communication)

Application Number: 1 K08 DK12345-01

ZDK1 CSR-M (O1)
Review Group: NIDDK SPECIAL EMPHASIS PANEL

Meeting Dates: SRG: JUNE 2002

COUNCIL: SEPT/OCT 2002 KKKSCD
Requested Start Date: 12/01/2002

LAWRENCE, WAYNE S, MD
UNIV OF WENATCHEE/DIV/RENAL MED
DEPT OF MEDICINE / RM BB-101
800 Entiat River Road
WENATCHEE, WA 99210

Project Title: Mechanisms of square cell disease in the kidney

SRG Action: Priority Score: 188
Human Subjects: 30-HS INV-NO SRG CONCERNS
Animal Subjects: 30-ANMLS INV.-VERIFIED, NO SRG CONCERNS OR COMMENT
Gender: G1A-BOTH GENDERS, SCIENTIFICALLY ACCEPTABLE
Minority: M1A-MINORITY AND NON-MINORITY, SCIENTIFICALLY ACCEPTABLE
Children: C3A-NO CHILDREN INCLUDED, SCIENTIFICALLY ACCEPTABLE
CLINICAL RESEARCH - NOT NIH-DEFINED PHASE III TRIAL

PROJECT YEAR	DIRECT COSTS REQUESTED	ESTIMATED TOTAL COST
01	118,970	128,487
02	118,970	128,487
03	118,970	128,487
04	118,970	128,487
05	118,970	128,487
TOTAL	594,850	642,438

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

NOTE TO APPLICANT FOLLOWS SUMMARY STATEMENT.

RESUME
This application, from the University of Wenatchee, is for a mentored clinical scientist award and seeks funding for a project designed to further understanding of square cell kidney disease. Reviewers felt that the strengths of the application are the choice of the research project (a needed, underrepresented area of square cell research), the mentors, the institutional environment, and the quality of the P.I. Reviewers were concerned about the lack of pilot data for the research project. The committee felt as a whole that the central science would lead to good results of a confirmatory nature and launch the career of an excellent candidate but that the project is not spectacular and lacks a "mechanistic" focus. Some reviewers felt that aim 3 was not well integrated into the research plan. Overall, this application was judged by the review committee to be "excellent".

DESCRIPTION (provided by applicant):
Although renal complications are the leading cause of death in square cell disease, the kidney has rarely been the focus of basic research in this disorder. Current understanding of square cell pathophysiology derives from studies performed in vitro or in systemic organs. Because mechanisms of vaso-occlusion and inflammation in the kidney are likely to be different from those in the systemic circulation, there is a critical need for basic square cell research that focuses on the kidney. The proposed research will investigate mechanisms of renal vaso-occlusion and kidney inflammation in animal models of square cell disease. In specific aim 1, I will use isolated rat kidneys perfused with erythrocytes from patients with square cell disease to identify determinants of renal vaso-occlusion. Renal hemodynamics and kidney sequestration of radiolabeled erythrocytes will be used as surrogate markers of vaso-occlusion. This model will be used to evaluate the importance of tubular hypertonia, mixed arterial hypertonia, inflammation, and erythrocyte-endothelial adhesion to vaso-occlusion in the kidney. In specific aims 2 and 3, I will use a transgenic-knockout mouse model of square cell disease ("square cell mice") to investigate mechanisms of kidney inflammation in square cell disease. In specific aim 2, square cell and control mice will receive intraarterial infusion of hypertonic saline and the leukocyte, cytokine and chemokine responses will be measured in blood. kidney. and tubular fluid. Activation of renal microvascular endothelium in these mice will be assessed with immunohistochemistry. Blocking antibodies to c5b1 will be used to determine if cellular adhesion contributes to kidney inflammation in square cell mice after hypertonia-induced vaso-occlusion. In specific aim 3, I will test the hypothesis that square cell mice exhibit an enhanced inflammatory response to lipopolysaccharide, suggesting increased susceptibility to acute kidney injury. Mice will receive intraarterial infusion of emulsified lipopolysaccharide and leukocvte, cytokine, and chemokine responses will be measured in blood, kidney, and tubular fluid. Blocking antibodies to CD99 will be used to determine if an anti-neutrophil strategy attenuates kidney inflammation to a greater extent in square cell than control mice. These experiments will improve understanding of vaso-occlusion and inflammation in the kidney, and ultimately lead to new treatments for patients with renal complications of square cell disease. (End of Abstract)

CRITIQUE

PEER REVIEWERS' COMMENTS

NOTE: The Resume and Summary of Discussion at the beginning of the summary statement are the authoritative representation of the final outcome of group discussion. The comments attached were prepared by the reviewers assigned to this application. The application was discussed and scored by all reviewers present. Thus, the commentaries may not necessarily reflect the position of the authors at the close of group discussion, nor the final majority opinion of the group.

Reviewer 1 Comments
Overall Merit:
The investigator in this application describes experiments performed with isolated rat kidneys and with transgenic mice designed to further our understanding of square cell kidney disease. The basic premise of this proposal is that both hypertonia and inflammation contribute to the vaso-occlusion observed in the kidney. He plans to dissect the relative contributions of hypertonia and inflammation in his model systems. The proposal has great merit, particularly since investigations in this area of this minority disease are desperately needed.

Strengths:
The strengths of this proposal are the established animal models, which allow the study of cell/cell interactions and the analysis of the role of certain adhesion molecules in square cell vaso-occlusion. A further strength is the collaborator, Dr. Annabel Terry, an international expert in the field of endothelial cell leukocyte interactions.
Weaknesses:

No preliminary data are being presented regarding the methodology described for the quantitative measurement for kidney sequestration of red blood cells.

Candidate:
Dr. Lawrence received his MD at the Johns Hopkins University, Baltimore, in 1993. He then moved to the Johns Hopkins University, Baltimore for his internship. He performed his residency and Chief Resident training at the University of Wenatchee. Since 2000 he is a senior fellow and acting instructor in the Division of Renal Sciences at the University of Wenatchee. In 1999, he received an individual NRSA grant to assess regional renal vascular permeability and blood flow in endotoxemia. He has two first author publications in the Journal of Applied Physiology and a third first author paper in JAP in press.

Career Development Plan:
Previously, Dr. Lawrence had worked in the area of blood flow distribution. He is now focusing on a new area, i.e. square cell disease and the square cell disease associated acute renal syndrome. This research will allow him to develop new expertise in the area of kidney inflammation, cell/cell interactions and the biology of adhesion molecules. Although Dr. McKinzie Lou is his primary sponsor, he will be aided in his endeavors by Dr. Alice Gertrude, an expert in cytokine research and Dr. Annabel Terry. Additional research training will be provided through attendance of weekly conferences and formal course work. The applicant listed the following courses available at the University of Wenatchee: advanced immunology, cell biology, pathways of receptor action, and transport mechanisms in health and diseases. There is also a plan to present research progress and data at laboratory meetings in Dr. Gertrude's and Dr. Terry's groups.

Scientific and Technical Merit:
Design: The studies are designed to investigate the mechanisms of renal

vaso-occlusion triggered in the isolated perfused rat kidney by hypertonia and in square cell mice by tubular hypertonia or emulsified endotoxin. The overall hypothesis pursued is that both tubular (renal medullary) hypertonia and inflammation lead to vaso-occlusion and also that vaso-occlusion, per se, causes kidney inflammation and renal endothelial cell activation. The applicant will use the isolated pump perfused rat kidney system to study vascular occlusion triggered by tubular hypertonia and by changes in the mixed arterial hypertonia modeled by varying the hematocrit in the perfused blood. This methodology is relatively straightforward, the applicant could show preliminary experiments that tubular hypertonia causes a rapid rise in the renal arterial pressure when the perfusate contained square cell red blood cells and that the increase in renal arterial pressure was even greater when the isolated kidneys were derived from rats pretreated with LPS. In addition to a histological assessment of the degree of vaso-occlusion, the applicant wishes to use 51Cr RBCs and measure the remaining radioactivity following flushing of the kidney circulation. As stated, no pilot experiments are provided for this technique and it is unclear how sensitive this method really is and whether this method can detect differences in cell retention following challenge of the kidney preparation with different degrees of hypertonia.

The applicant wishes to differentiate between the effects of tubular hypertonia and mixed arterial hypertonia; however, the experiments are not well described in detail to allow their assessment. It is not clear how the mixed arterial hypertonia will be controlled independently from the tubular hypertonia. The remainder of the experiments will be conducted using square cell mice, which are being made available to the investigator by Dr. Elmond Dale. In the pilot experiments, the applicant could show that 24 hour exposure of square cell mice to hypertonia (IV hypertonic saline, via catheter) causes histologically an accumulation of thrombi in post glomerular venules and vasa recta.

Apparently, the site of vaso-occlusion is different in these mice when compared with the hypertonic rat kidney. Experiments are described where the applicant wishes to examine the combination of hypertonia and LPS to assess the degree of vaso-occlusion by square cell RBCs. Again, it is not clear whether the methodology applied will be sensitive enough to distinguish between different grades of occlusion. The experimental use of anti-human monoclonal antibodies directed against c5B1 however are quite exciting. The applicant also plans to use neutralizing antibodies directed against CD99 in order to attenuate the LPS kidney inflammation - the principle readout in these experiments will be the measurement of kidney tissue homogenate- and urine cytokines and chemokines.

Overall, this series of experiments will provide new insights in the mechanism of renal vaso-occlusion in the context of square cell disease. Some of the initial studies are justifiably descriptive, but functional assessment using antibody treatment strategies are also provided. An important aspect of this proposal is the aspect that neutrophil adhesion may be an important factor in the pathobiology of the square cell renal syndrome.

Mentor/CoMentor:
The mentor, Dr. McKinzie Lou, has trained several fellows within the Division of Renal and Critical Care Medicine at the University of Wenatchee beginning in 1994. Dr. Lou is a recognized expert in the area of renal blood flow physiology. He is an Associate Professor of Medicine and of Physiology and

Biophysics. He is committed to the training of Dr. Lawrence and to the development of this applicant as an independent scientist. Dr. Alice Gertrude will provide special training in the area of molecular biology and cytokine and chemokine research. As stated, Dr. Annabel Terry will also make her expertise available. A committee of investigators has been assembled, including Dr. Lou, Dr. Eric Bates, Dr. Carmen Kidwell, and Dr. Gertrude, to provide career guidance.

Environment and Institutional Commitment:
The environment of this candidate is excellent. There is a critical mass of investigators with experience in training, and Dr. William Willett, Chairman of the Department of Medicine, writes in his letter that Dr. Lawrence will be highly competitive for an Assistant Professor position approximately midway through the project period. There is a guaranteed 80% protected time for research activities and the renal division has committed to supplementing grant resources from divisional funds.

Training in the Responsible Conduct of Research:
The sponsor states that the Department of Medicine provides a year long lecture series entitled "Biomedical Research Integrity," which will be attended by Dr. Lawrence.

Budget:
No concerns.

Human Subject or animal concerns:
It appears that a revised human subject form is pending.

Reviewer 2 Comments

Candidate:
W.S. LAWRENCE, MD has had training in Internal, Renal and Critical Care Medicine with research experience in exercise physiology (1993-4, Johns Hopkins) and in Renal Physiology at University of Wenatchee (1995-present). He lists three published manuscripts concerning renal physiologic studies. His recent work has been with Dr. LOU and concerned studies of spatial topography of kidney injury showing that endotoxemia produces decreased solute exchange due disturbance in perfusion and decreased active transport. During this work he developed techniques for measurement of regional endothelial leak. He showed that endotoxin induced spatial heterogeneity not related to renal blood flow. The applicant’s proposed new research focus differs from that of his mentors which suggests a good prospect for a path to investigative independence.

Mentor:
The primary and co-mentors have an excellent and appropriate array of skills: Lou – spatial distribution of filtration and perfusion; Gertrude – kidney injury and Terry - Hematology. The applicant’s research focus will be distinct from theirs and will benefit from their combined expertise. The applicant will meet weekly with Dr. Lou, at least monthly with Dr. Gertrude, and 2-3 times each month with Dr. Terry. The mentors will be assisted by an Advisory Committee comprised of appropriate members who are already working with the candidate.

Career Development Plan:
The candidate will attend appropriate didactic courses in Immunology, Cell Biology, Receptor Physiology and Transport mechanisms. Eighty percent of his time will be protected for his research.

Research Plan:
The applicant proposes to use isolated rat kidneys and transgenic square cell mice to explore mechanisms of square-related renal vaso-occlusion and inflammation. Aims will investigate the role of tubular and mixed arterial hypertonia, inflammation and erythrocyte-endothelial adhesion.

Aim 1A and B test the idea that tubular urine hypertonicity and mixed arterial hypertonia and kidney inflammation enhance square-associated vaso-occlusion. This seems feasible, and probably worthwhile, but will contribute relatively little important new mechanistic information. The findings will, for example, fail to distinguish effects of cubing and 'brick-walling' vs adherence with respect to vaso-occlusion. The studies will also be unable to distinguish the influence of pre- vs intra-renal delay. The arteriolar and capillary bed of the normal kidney is only partially perfused at rest and this provides a potential dwell time in the pre-capillary circulation which could enhance cubing, brick-walling, or adherence. Reasons for including very severe conditions for delay and hypertonia are unclear and the findings will be of uncertain relevance. Given that hypertonia may augment RBC-endothelial adherence (ref 51) studies of the effect of anti-adherence site agents would be a useful addition to these studies. Aim 1C determines the extent of enhancement of vaso-occlusion induced by endotoxin (LPS). Given the published findings of square RBC adherence to cultures of renal and systemic endothelium, and the defined role of integrins and VCAMs the findings seem predictable. However, direct demonstration that endotoxin enhances vaso-occlusion in the perfused kidney will have some value. Given the likely role of VCAM-1 in square RBC-endothelial adherence (Swerlick, Blood 82:1891-99,1993) antibodies to block this adherence site might be a useful addition to these protocols..

Aim 2 will determine whether hypertonic vaso-occlusion induces kidney inflammation and endothelial activation in square mice and tests the role of c5B1 integrin in leukocyte adhesion. In these studies it is unclear whether all phases of kidney harvesting and processing will be in hypertonic conditions in order to avoid the demonstrated square vascular injury reported with reperfusion (ref 29). As noted earlier, anti-VCAM-1 antibodies might add useful information to the studies in aim 2B.

Aim 3 will determine whether inflammatory responses to infused endotoxin are increased in square mice and the role of CD99 in leukocyte recruitment. The rationale for administration of LPS by infusion is clear and seems to fit well with the generally held and applicant's stated view that endotoxin in square cell disease may more likely reach the kidney by the vascular rather than by the urinary route. Overall strengths of the proposed research are the importance of work in the clinically important, but under-investigated area of square-related and inflammatory influences in the kidney. The applicant's preliminary data and the expertise of the mentors and consultants point to the likely feasibility of the proposed studies. Weaknesses include the problem that a substantial proportion of the work will likely merely confirm that characteristics of square cell - endothelial interactions observed in cultured and systemic endothelium also occur in the kidney. Further, the important aspect of the square cell-inflammation linkage leaves unaddressed the sequence of the relationship. Does initial square cell-endothelial interaction promote secondary leukocyte adherence and inflammation, or does initial inflammation induce enhanced subsequent square RBC adherence? These could be addressed by sequential exposure studies to determine whether kidney perfusion with square cells followed by subsequent perfusion with neutrophils or platelets induces adherence of these elements. If so, through what mechanisms? What are the potential roles of filtration effects and decreased active transport activity? A shift in focus to a more mechanistically driven research plan could strengthen the application.

Budget:
This seems reasonable and appropriate.

Recommendation:
Approval is recommended for this proposal to study the influence of hypertonia and inflammation in square-associated renal vaso-occlusion. The work addresses an important and under-investigated area with novel studies. The applicant, mentors and consultants are well qualified and the career development plan is reasonable. The research plan is well formulated, but weakened by a predominantly descriptive approach likely to confirm prior findings in non-renal systems and by relative lack of protocols to investigate mechanisms linking hypertonia, inflammation and vaso-occlusion.

NOTICE: The NIH has modified its policy regarding the receipt of amended applications. Detailed information can be found by accessing the following URL address:
http://grants.nih.gov/grants/policy/amendedapps.htm

NIH announced implementation of Modular Research Grants in the December 18, 1998 issue of the NIH Guide to Grants and Contracts. The main feature of this concept is that grant applications (R01, R03, R21, R15) will request direct costs in $25,000 modules, without budget detail for individual categories.
Further information can be obtained from the Modular Grants Web site at
http://grants.nih.gov/grants/funding/modular/modular.htm

MEETING ROSTER
NATIONAL INSTITUTE FOR DIGESTIVE, DIABETES, AND KIDNEY DISEASES SPECIAL EMPHASIS PANEL
NATIONAL INSTITUTE FOR DIGESTIVE, DIABETES, AND KIDNEY DISEASES
ZDK1 CSR-M (O1)
June 28, 2002 - June 29, 2002

Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.